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# Sublethal Effects of Toxaphene on Daphnids, Scuds, and Midges

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Daphnids (Daphnia magna), scuds (Gammarus pseudolimnaeus), and midge larvae (Chironomus plumosus) were continuously exposed to toxaphene in a flow-through system. Exposure of daphnids for a complete life cycle (21 days) to 0.12, 0.28, 0.54, and 1.0 ug/l of toxaphene significantly ($P<0.05$) reduced production of young; the no-effect concentration was 0.07 ug/l. Toxaphene concentrations of 0.25 ug/l and greater significantly ($P<0.05$) reduced growth of scuds and concentrations of 3.2 ug/l and greater significantly ($P<0.05$) reduced emergence of midges. The no-effect concentrations were 0.13 ug/l for growth of scuds and 1.0 ug/l for emergence of midges. Daphnids continuously exposed to toxaphene accumulated residues after 7 days that were 4,000 times (based on organism wet weight) and water concentration of 0.06 ug/l. Whole body residues in midge larvae were below the minimum detection limit of 0.1 ug/g. Maximum acceptable toxicant concentrations (MATC) of toxaphene for the three species of aquatic invertebrates were estimated using reproduction of daphnids, growth of scuds, and emergence of midges as indicators of toxic effects. The MATC was estimated to be between 0.07 and 0.12 ug/l for daphnids, between 0.13 and 0.25 ug/l for scuds, and between 1.0 and 3.2 ug/l for midges.

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# SUBLETHAL EFFECTS OF TOXAPHENE ON DAPHNIDS, SCUDS, AND MIDGES

by

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Contract No. EPA-IAG-141(D)

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ENVIRONMENTAL RESEARCH LABORATORY
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## FOREWORD

This report describes the toxicity of a pesticide, toxaphene, to three species of aquatic invertebrates. Toxaphene has diverse uses; as a piscicide for controlling fish populations; an insecticide for controlling insect pests on livestock; most extensive use is on agricultural lands as an insecticide on crops.

Toxaphene is persistent and highly toxic to aquatic organisms but little is known about the chronic effects on growth or reproduction. Research reported here helps determine chemical pollution affects to aquatic life and bioaccumulation in aquatic organisms.

This report studies the effects of the insecticide toxaphene on reproduction in daphnids (Daphnia magna); on growth in length of scuds (Gammarus pseudolimnaeus); and on emergence of midges (Chironomus plumosus); when continuously exposed to five different concentrations.

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## ABSTRACT

Daphnids (Daphnia magna), scuds (Gammarus pseudolimnaeus), and midge larvae (Chironomus plumosus) were continuously exposed to toxaphene in a flow-through system. Exposure of daphnids for a complete life cycle (21 days) to 0.12, 0.28, 0.54, and 1.0 ug/l of toxaphene significantly ($P<0.05$) reduced production of young; the no-effect concentration was 0.07 ug/l. Toxaphene concentrations of 0.25 ug/l and greater significantly ($P<0.05$) reduced growth of scuds and concentrations of 3.2 ug/l and greater significantly ($P<0.05$) reduced emergence of midges. The no-effect concentrations were 0.13 ug/l for growth of scuds and 1.0 ug/l for emergence of midges. Daphnids continuously exposed to toxaphene accumulated residues after 7 days that were 4,000 times (based on organism wet weight) the water concentration of 0.06 ug/l. Whole body residues in midge larvae were below the minimum detection limit of 0.1 ug/g. Maximum acceptable toxicant concentrations (MATC) of toxaphene for the three species of aquatic invertebrates were estimated using reproduction of daphnids, growth of scuds, and emergence of midges as indicators of toxic effects. The MATC was estimated to be between 0.07 and 0.12 ug/l for daphnids, between 0.13 and 0.25 ug/l for scuds, and between 1.0 and 3.2 ug/l for midges.



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# SECTION 1

# INTRODUCTION

Toxaphene (technical chlorinated camphene containing 67-69% chlorine) is a synthetic organochlorine insecticide that contains numerous isomers. It has been used as a piscicide for controlling fish populations in lakes[1, 2], including sea lamprey populations in the upper Great Lakes[3] and for controlling insect pests on livestock[4]. However, its most extensive use has been on agricultural lands for controlling a variety of insect pests on cotton, grains, fruits, and forage. Of the more than 30 million pounds of this insecticide used annually in the United States, over half is applied for controlling insect pests on cotton[4].

Toxaphene is a complex compound and a highly persistent chemical in the aquatic environment[5, 6]. It has been reported to persist in hydrosoil at concentrations of 0.2 to 1 mg/kg for over 6 years[7]. Since many aquatic invertebrates live in or on the surface of the hydrosoil, the presence of low toxaphene concentrations may reduce growth and inhibit reproduction, which in turn may affect fish and other animals that feed on these organisms.

Only limited data have been published concerning the effects of toxaphene on aquatic invertebrates and most of the information has resulted from observations following field application of this chemical in fish control projects and fish population studies[8-10]. Laboratory studies with toxaphene and aquatic invertebrates have been concerned primarily with acute toxicities[11-13], and few data are available on the chronic effects of the compound on reproduction, metamorphosis, or growth. Since the biological significance of toxaphene residues in aquatic invertebrates is largely unknown, the present study was undertaken to evaluate the effects of toxaphene on reproduction in daphnids (Daphnia magna); on growth in length of scuds (Gammarus pseudolimnaeus); and on emergence of midges (Chironomus plumosus). These organisms make up a significant portion of the macroinvertebrate fauna in many freshwater habitats, and they are important food of many species of fish and waterfowl.

## SECTION 2

## CONCLUSIONS

Daphnid reproduction was significantly ($P<0.05$) reduced at toxaphene concentrations of 0.12 µg/l or higher, but not at 0.07 µg/l.

Growth of scuds was significantly ($P<0.05$) decreased at toxaphene concentrations of 0.25 µg/l or higher; the no-effect concentration was 0.13 µg/l.

Continuous exposure of midge larvae through a complete life cycle to toxaphene concentrations of 3.2 µg/l and higher significantly ($P<0.05$) reduced pupation and emergence; the no-effect concentration was 1.0 µg/l.

Toxaphene residues accumulated by daphnids during a 7-day exposure were 4000 times (based on organism wet weight) the water concentration of 0.06 µg/l.

In contrast midge larvae accumulated little or no toxaphene; whole body residues were below the minimum detection limit of 0.1 µg/g.

Reproduction of daphnids was the most sensitive indication of invertebrate species susceptibility to chronic exposure to toxaphene.

Based on chronic tests evaluating reproduction of daphnids, emergence of midges, and growth of scuds, the maximum acceptable toxicant concentration was estimated to be between 0.07 and 3.2 µg/l.

## SECTION 3

## RECOMMENDATIONS

Inasmuch as reproductive impairment in daphnids was found to be a sensitive criterion for evaluating the chronic toxicity of toxaphene to an aquatic invertebrate, Daphnia magna should be used in future chronic toxicity studies until methods for culturing and testing other species of daphnids can be developed.

Although length measurement, used in this study for determining growth, was found to be a sensitive method for evaluating the chronic toxicity of toxaphene to the scud Gammarus pseudolimnaeus, further research on laboratory rearing of this organism is needed. Techniques for maintaining a healthy reproducing population of scuds through a complete life cycle must be improved.

The midge Chironomus plumosus should be considered as a test organism in chronic toxicity studies because it adapts well to laboratory conditions, is widely distributed in a variety of aquatic habitats, has easily recognizable development stages, and is an important food of young and adult fish.

## SECTION 4

## MATERIALS AND METHODS

Test organisms included two crustaceans and an aquatic insect; a daphnid (Daphnia magna); a scud (Gammarus pseudolimnaeus); and the larvae of a midge (Chironomus plumosus). The rearing method described by Sanders and Cope[11] was used in maintaining a continuous supply of daphnids. Scuds were from cultures maintained in the laboratory[14]. Rearing techniques described by Biever[15] and Ivlera[16] were used to maintain a continuously reproducing population of midges. Daphnids and scuds were tested at 18 ± 1 C and midge larvae at 22 ± 1 C. A combination of Duro-test and wide-spectrum Growlux bulbs provided light over the cultures and tests. The photoperiod was automatically controlled for 16 hours light and 8 hours dark.

### WATER CHARACTERISTICS

The water used for cultures and all toxicity tests was from a deep well. Chemical characteristics of this water are summarized in Table 1. During the chronic exposure, the dissolved oxygen concentration and water temperature were measured daily in test containers. The pH and hardness were measured at the beginning and termination of each test.

### TOXICANT INFORMATION

An experimental-use sample of toxaphene (X-16189-49) was furnished by Hercules Inc. Stock solutions of toxaphene were prepared in ethanol and further diluted with water in a flow-through system modeled after Mount and Brungs[17], and were delivered by the apparatus designed by Chandler et al.[18]. All concentrations, as well as the controls, contained 0.1 ml/l of ethanol.

Prior to initiating the tests, the flow-through systems were operated for 24 h to allow for concentration equilibrium and to establish that toxaphene water concentrations were constant. Toxaphene concentrations in water were measured in the high, medium, low, and control containers before the introduction of test organisms and at termination of the tests. Methods used for water residue analysis were described by Stalling and Huckins[19].

### ACUTE TOXICITY PROCEDURES

Acute toxicity tests were conducted under static conditions; methods used were those recommended for standardized laboratory toxicity tests by the Committee on Methods for Toxicity Tests with Aquatic Organisms[20]. Daphnids were first instar; scuds in an early instar; and midges in the early fourth instar. The measure of acute toxicity for daphnids and midge larvae was

TABLE 1. CHEMICAL CHARACTERISTICS OF WELL WATER AT THE COLUMBIA NATIONAL FISHERIES RESEARCH LABORATORY

| Parameter | Specified sensitivity limits, mg/liter | Concentration, mg/liter |
|---|---|---|
| Ca | 0.1 | 70 |
| Mg | 0.1 | 27 |
| K | 0.5 | 3.9 |
| $SO_4$ | 0.01 | 4.4 |
| $NO_3$ | 0.05 | <0.05 |
| $NO_2$ | 0.05 | <0.036 |
| $NH_4/N$ | 0.01 | 0.066 |
| Phenol | 0.001 | <0.001 |
| $Cl_2$ | 0.001 | <0.001 |
| Cl | 0.01 | 29 |
| F | 0.01 | 0.34 |
| CN | 0.005 | 0.006 |
| Fe | 0.01 | 0.014 |
| Cu | 0.0001 | 0.0045 |
| Zn | 0.001 | <0.001 |
| Cd | 0.001 | <0.0005 |
| Cr | 0.01 | <0.01 |
| Pb | 0.001 | 0.0015 |
| Alkalinity | 1.0 | 237 |
| Hardness (EDTA) | 1.0 | 272 |
| pH | 0.1 | 7.4 |

the 48 h median effective concentration (48-h EC50) based on immobilization. The toxicity of toxaphene to scuds was expressed in terms of LC50, the calculated concentration of chemical in water which produces a 50% mortality of test organisms during a specific time.

## CHRONIC TOXICITY PROCEDURES

### Reproduction of Daphnids

The daphnid reproduction studies were begun by introducing 10 first-instar daphnids up to 24 h old into duplicate exposure vessels containing 1 liter of water. Thus, 20 daphnids per treatment were exposed continuously through a complete life cycle (21 days) to toxaphene concentrations of 0, 0.07, 0.12, 0.28, 0.56, or 1.0 ug/1. Organisms were fed a suspension of yeast in sufficient amounts to support a stable population. Reproductive success was determined by counting the offspring produced in each concentration after the parent daphnids had been exposed for 21 days. The mean number of young produced per adult was determined by averaging the number of young produced in two replicate tests. Data were analyzed by analysis of variance, and significant differences among treatments were determined by a multiple means comparison test (least significant difference[21]).

### Growth of Scuds

The study of chronic toxicity of toxaphene to scuds was started with young collected from gravid females. Ten young scuds were placed in each container and fed maple leaves that had been previously soaked in water for several weeks. The scuds (5-10 days old) were exposed to toxaphene for 30 days in a flow-through system[17]. Flow-splitting chambers designed by Benoit and Puglisi[22] were used to mix and divide each toxaphene concentration into four exposure chambers. The average measured concentrations were 0, 0.06, 0.13, 0.25, 0.50, and 1.0 ug/1.

A method proposed by McKim and Benoit[23] for measuring lengths of juvenile fish from photographs was used to measure growth of the scuds. Scuds were photographed at 0 and 30 days of exposure. Measurements were made on the photo-enlarged image of the scuds, and lengths of animals were determined from interpretation of the photographs.

### Emergence of Midges

The study of chronic toxicity of toxaphene to midge was started with first-instar larvae (1.5 mm long and up to 24 h old). One hundred larvae, counted with the aid of a 10X lens, were placed in duplicate exposure contaminers that had been previously prepared by adding 13 g of sand and 0.3 g of a commercial dog candy[15] to 1 liter of water. The larvae were exposed to toxaphene concentrations of 0, 1.0, 3.2, 5.6, 10, and 32 ug/1. During the test larvae were fed 0.3 g of the candy every 5 days until they transformed into the pupal stage. The test was ended after 30 days, when about 80-95 percent of the control larvae had completed metamorphosis into the adult form. Cast pupal skins at the water surface in test containers were counted and removed daily to determine adult emergence. The effects of toxaphene on midge emergence

were determined by conducting an analysis of variance on the arcsin transformation for portions (angle = arcsin $\sqrt{\text{percentage}}$) followed by a least significant difference test[21].

Residue Accumulation

Accumulation studies with daphnids and midge larvae were conducted in a flow-through system; the toxaphene was delivered with the apparatus described by Chandler et al.[18]. Daphnids were exposed to measured toxaphene concentrations of 0.06 and 0.12 ug/l and midge larvae to concentrations of 0.25, 1.8, and 3.2 ug/l. These concentrations, selected on the basis of the results of the chronic-toxicity studies, represent the lowest toxaphene concentration that produced an effect on the organisms and a concentration in which no effect would be expected. Daphnids and midge larvae (500 mg of each) were sampled from each concentration after 1, 7, and 14 days of exposure. Residue analyses were performed according to the method of Stalling and Huckins[19]. Samples were prepared for analysis by homogenizing 100-500 mg of organisms with 8 g of anhydrous sodium sulfate. The samples were extracted by percolation in 1 cm i.d. glass columns with 50 ml portions of 5% diethyl ether in petroleum ether. Sample cleanup was accomplished by adding the sample extract to 2 g of heated Florisil in a 1 cm i.d. column and eluting toxaphene with 45 ml of 5% diethyl ether in petroleum ether. The samples were concentrated to 0.5 ml and toxaphene residues were quantified by gas liquid chromatography (GLC) with $^{63}$Ni-electron capture detection. A 160 cm long x 2.0 mm i.d. glass column packed with 3% (w/w) OV-7 on Chromosorb W-H.P. was used, with a 30 ml/min flow of nitrogen carrier gas. The column was operated at 200 C. The minimum detection limit of a toxaphene standard was 0.5 ng. For sample quantities of 300-500 mg the detection limit was 0.1 ug/g.

CALCULATION OF APPLICATION FACTORS

A method proposed by Mount and Stephen[24] for establishing acceptable toxicant limits for aquatic organisms under continuous exposure conditions was used to calculate an application factor for determining a toxicant concentration that has no adverse effect on reproduction of daphnids, emergence of midges, or growth of scuds. The application factor consists of the laboratory determined maximum acceptable toxicant concentration (MATC) that has no effect on the test organisms during chronic exposure, divided by the 48-h EC50 for daphnids and midges and the 96-h LC50 for scuds.

## SECTION 5

## RESULTS

### ACUTE TOXICITY

In static tests the toxicity of toxaphene varied widely among the invertebrates tested, with the crustacea being the more susceptible. The 48-h EC50's ranged from 10 ug/l for daphnids to 180 ug/l for midges. The 96-h LC50 for scuds was 24 ug/l.

### REPRODUCTION OF DAPHNIDS

Continuous exposure of daphnids through a complete life cycle (21 days) to toxaphene concentrations of 0.12, 0.28, 0.54, and 1.0 ug/l significantly reduced ($P<0.05$) production of young (Table 2). Production of young in the 0.7 ug/l concentration was similar to that in controls after 21 days of exposure. Survival of adult daphnids at the end of the tests was 90 - 95% in all toxaphene concentrations and controls, except at 1.0 ug/l, in which adult survival was 70%.

The 48-h EC50 of 10 ug/l for first-instar daphnids was approximately 80 times greater than the lowest toxaphene concentration (0.12 ug/l) that affected daphnid reproduction. Based on the chronic exposure of daphnids to toxaphene, the MATC was estimated to be between 0.07 and 0.12 ug/l and the application factor was between 0.007 and 0.01.

### GROWTH OF SCUDS

Growth of scuds as measured by increases in length, was significantly decreased ($P<0.05$) in toxaphene concentrations of 0.25, 0.50 and 1.0 ug/l after 30 days of exposure. Growth of scuds at 30 days was similar in controls and toxaphene concentrations of 0.06 and 0.13 ug/l. Survival after the 30 day exposure was 90-100% in all concentrations and controls. However, data on survival of scuds beyond 30 days were too variable for sound statistical analyses.

The 96-h LC50 of 24 ug/l for scuds was about 180 times greater than the lowest toxaphene concentration (0.13 ug/l) that affected scud growth. The MATC for scuds was estimated to be between 0.13 and 0.25 ug/l and the application factor was between 0.001 and 0.01.

TABLE 2. EFFECTS OF DIFFERENT CONCENTRATIONS OF TOXAPHENE ON REPRODUCTION OF *DAPHNIA MAGNA* AFTER CONTINUOUS EXPOSURES OF 14 AND 21 DAYS AT 18 ± 1 C.

| Toxaphene concentration (µg/l) | Number of offspring after: 14 days | 21 days |
|---|---|---|
| 0 | 308 | 596 |
| 0.07 | 310 | 542 |
| 0.12 | 186[a] | 289[a] |
| 0.28 | 165[a] | 212[a] |
| 0.54 | 100[a] | 154[a] |
| 1.0 | 76[a] | 110[a] |

[a]Significantly different from controls ($P<0.05$), n = 2.

## EMERGENCE OF MIDGES

Emergence of midges was significantly reduced ($P<0.05$) after 30 days exposure to toxaphene concentrations of 3.2, 5.6, 10, and 32 ug/l (Table 3). Emergence was also significantly delayed ($P<0.05$) in the 1.0 ug/l concentration at 20 days; however, at 30 days the emergence time was similar to that in the controls. Pupation was progressively reduced and the length of development was increased in concentrations of 5.6 ug/l through 32 ug/l. Larvae held at these concentrations behaved abnormally and appeared unable to build well defined larval tubes, within which pupation occurs. At the end of the study, some of these larvae had not pupated, and appeared to be stunted. This reduction in pupation was directly related to the reduction in emergence of adults at the higher concentrations.

The 48-h EC50 of 180 ug/l was about 60 times greater than the lowest toxaphene concentration (3.2 ug/l) that significantly reduced emergence. The MATC for midges was estimated to be between 1.0 and 3.2 ug/l and the application factor was between 0.005 and 0.01.

## RESIDUE ACCUMULATION

The magnitude of toxaphene accumulation from water by daphnids during continuous exposure was proportional to the toxaphene concentration in water. After a 7 day exposure to a measured toxaphene concentration of 0.12 ug/l, daphnids concentrated toxaphene 4,000 times (0.5 ug/g; wet weight) the level in water. When daphnids were exposed to 0.06 ug/l of toxaphene, they accumulated total body concentrations 4,000 times (0.25 ug/g) that of water. Uptake at both toxaphene concentrations reached a peak after 7 days of exposure. However, residues in daphnids did not stabilize at the peak concentration with additional exposure (14 days); rather, the residues were eliminated at rates exceeding the accumulation rate. Residues in daphnids exposed at 0.12 ug/l declined by 50 percent between 7 and 14 days, but residues in those exposed at 0.06 ug/l declined by only 10 percent.

When midge larvae were exposed continuously to toxaphene concentrations in water of 1.8, 3.2, and 5.6 ug/l for 1, 7, and 14 days, whole body residues in all samples remained below the minimum detection limit of 0.1 ug/g for tissues. These results indicate that the sample size (500 mg sample of larvae, representing 250 larvae) was not adequate for residue analyses. It was not feasible to expose a larger population of larvae because of problems in rearing large numbers of organisms. Nevertheless, these negative data suggest that midge larvae accumulate far less toxaphene than daphnids.

TABLE 3. CUMULATIVE PERCENTAGES OF MIDGES (CHIRONOMUS PLUMOSUS) THAT EMERGED AFTER CONTINUOUS EXPOSURE OF THE LARVAE TO DIFFERENT CONCENTRATIONS OF TOXAPHENE AT 22 ± 1 C.

| Days of exposure | Toxaphene (µg/1) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 1.0 | 3.2 | 5.6 | 10 | 32 |
| 15 | 9 | 0 | 0 | 0 | 0 | 0 |
| 20 | 66 | 41[a] | 34[a] | 20[a] | 26[a] | 12[a] |
| 25 | 86 | 81 | 54[a] | 42[a] | 42[a] | 20[a] |
| 30 | 88 | 82 | 56[a] | 43[a] | 44[a] | 20[a] |

[a]Significantly different from controls ($P<0.01$), n = 2.

## SECTION 6

## DISCUSSION

Aquatic invertebrates are often exposed to toxaphene applied directly to their habitat[5], or as a result of runoff from treated agricultural lands. Observations of various aquatic invertebrates after field application of toxaphene confirm the response of these organisms to exposure to this chemical. Hilsenoff[8] reported that the application of 100 ug/l of toxaphene for control of rough fish in a lake resulted in total elimination of the midge population and that midge larvae did not reappear until 9 months after treatment. Results from the present study suggest that reductions of a midge population might occur at much lower concentrations, since a toxaphene concentration of 3.2 ug/l would cause a significant reduction in pupation and emergence of midges.

Laboratory studies have shown that toxaphene is acutely toxic to fish (96-h LC50's = 2.0 to 14 ug/l[25, 26]), whereas daphnids are slightly more resistant. Sanders and Cope[11] reported that the 48-h EC50 immobilization values for three species of daphnids ranged from 10 to 19 ug/l[11]. These results tend to support the observations made by Tanner and Hayes[9], who reported that a lake treated with this chemical for rough fish control supported large populations of daphnids several weeks after treatment but, remained extremely toxic to fish. This greater resistance of daphnids to toxaphene could enable them to concentrate significant residues and pass these residues through the food chain to higher trophic levels. This conclusion also agrees with the findings of Schoettger and Olive[27], who reported that static exposure of daphnids to toxaphene concentrations of 10-20 ug/l for 312 h did not produce detrimental effects. However, sufficient residues were accumulated by the daphnids during this exposure to produce complete mortality in test fish fed these exposed organisms. The results of our uptake studies with daphnids indicated that maximum accumulation occurred during the first 7 days of exposure. Residues in daphnids exposed at a concentration of 0.12 ug/l of toxaphene did not attain a stable equilibrium and residues declined by half between 7 and 14 days of continuous exposure; however, residues in daphnids exposed at a concentration of 0.06 ug/l of toxaphene declined only slightly. This relation suggests that the factors that contribute to metabolism and excretion may have been stimulated at the 0.12 ug/l concentration and caused the elimination rate to exceed the accumulation rate. Although the residues accumulated by daphnids were not identified, it is assumed the loss was caused by excretion and degradation of the parent compound. Mayer et al.[28] reported that toxaphene was degraded in fish and that the more chlorinated toxaphene isomers are preferentially stored by brook trout (Salvelinus fontinalis) while the less chlorinated ones are more rapidly eliminated. Further studies are needed to determine residue data from

various components of similated or natural food chains.

Chronic toxicity determined for the three species of aquatic invertebrates indicated that toxaphene is biologically active at concentration's well below those that are acutely toxic. All organimsm were susceptible to chronic exposure to toxaphene and the no-effect concentrations ranged from 0.07 to 1.0 ug/1. On the basis of chronic tests evaluating reproduction of daphnids, emergence of midges, and growth of scuds, the maximum acceptable toxicant concentration (MATC) of toxaphene was estimated to be between 0.07 and 3.2 ug/1. Dividing the approximate MATC by the appropriate EC50 or LC50 gives an application factor ranging from 0.001 to 0.01. This range in values approximates the application factor of 0.01 which has been used for establishing water quality criteria for organochlorine insecticides[29].

Fishes appear to be more susceptible to toxaphene than the aquatic invertebrates tested; the maximum toxaphene concentration in water acceptable for brook trout fry, determined by Mayer et al.[28] to be below 0.039 ug/1, should be relatively safe for these freshwater organisms.

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